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Full Length Research Paper

Phytochemical and antimicrobial properties of leaves of African mistletoe (*Loranthus micranthus*) on some selected microbial pathogens in Abia State, Nigeria.

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In this study performed on the leaves African mistletoe (*Loranthus micranthus*), phytochemicals such as Tanins, Saponins, Flavonoids, Phenols, Alkaloids and Anthrocyanins were quantitatively determined. Composition studies indicated the presence of calcium, magnesium, phosphorus, sodium and potassium at different concentrations. Antimicrobial susceptibility was observed at various concentrations (50mg/disc – 300mg/disc) on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus* species, *Penicillium* species. Susceptibility variance should be stated. Highest – lowest. No antibacterial activity was observed on *Salmonella typhi* at concentration <300mg/disc. Minimum inhibitory concentrations (MIC) were determined with different organisms and results documented. This research calls for further pharmacological research attention on *Loranthus micranthus* with the view to formulating some refined therapies from this herb.

Keywords: Phytochemicals, Antimicrobial, African Mistletoe, Ethanollic Leaf Extract, Abia State, Nigeria.

INTRODUCTION

There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing countries (Murray, 1998).

The need for more potent, safe and affordable drugs has led to intensified research into herbal drugs, the result of which is the introduction of new herbal preparation for therapeutic uses (Griggs, 1991).

African mistletoe (*Loranthus micranthus*) belongs to the family Loranthaceae, and it is an obligate semi-parasitic

plant of *Kola acuminator*, *Baphia nitida*, *Citrus limon*, and *Pentaclethra macrophylla*.

It is commonly found in the rain forest region of Nigeria and other parts of Africa, Europe and America. It is among the plants whose leaves are used for pharmaceutical practices (Kernezehok et al., 1997).

Medicinal plants contain accumulated natural products, biologically active materials and ingredients which have various effects on humans, and microorganisms. The plant part that contains the active ingredient is taken in the form of extract, infusion and decoction (Hornok, 1992).

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It has been reported in recent time that 50% of top pharmaceutical products sold are linked to natural product research (Falodun et al., 2007).

Mistletoe preparations are commonly used in complementary medicine as anticancer agents (Grossarth et al., 2007), antidiabetic agents (Osadebe et al., 1998), bacteriostatic agent (Fulder, 1998) and antihypertensive agent (Nkanu et al., 2003).

The herb has been analyzed and observed to contain Lecithin, Viscotoxin, Polysaccharides and many Phytochemicals as active ingredient (Leeper, 1999).

Loranthus micranthus has been used in the treatment of arteriosclerosis, AIDS, Skin Problems, such as acceleration of skin ageing (Bonnia et al., 1998; Nweze et al., 2004). It has been reported to have hypoglycemic properties because it decreases the blood glucose level and has effects of controlling the loss of body weight which occurs during diabetes milletus (Obatomi et al., 1994; Akinjobi et al., 2004). The mistletoe plant has also shown Broad spectrum of antimicrobial properties against certain drug resistant bacterial and fungal organisms of farm animals (Deen et al., 2002).

This plant has also been used in the effective treatment of epilepsy, infertility in men and women, menopausal syndrome and rheumatism (Osadele and Okwueze, 2004).

In this study the quantities of phytochemicals and mineral elements present in the *Loranthus micranthus* under study was investigated the zones of inhibition of the different concentrations of its leaf extracts on different microbial pathogens and minimum Inhibition Concentration (MIC) of *Loranthus micranthus* were determined.

MATERIALS AND METHOD

Collection of Plants

The leaves of *Loranthus micranthus* were collected from different endemic regions in Akanu Town, Ohafia Local Government Area of Abia State, and the plant leaves were identified by a team of Botanists at the National Root Cross Research Institute, Umudike – Umuahia, Abia State of Nigeria.

Test Microorganisms

Fresh cultures of the microorganisms which were maintained in agar slants for bacterial organisms and fungal organisms were used.

The density of the microorganisms was adjusted as per McFarland 0.5 Standard. The microorganisms have already been characterized and identified in different Reference Hospitals in Abia State, Nigeria. Total of seven (7) microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida*

albicans, *Aspergillus* species and *Penicillium* species were used.

These organisms were obtained from the Reference Hospitals where they were characterized and identified as shown in tables 5 and 6.

Quantitative determination of phytochemical and mineral elements.

The leaves were washed and dried, and later placed under oven at 65°C. At the point of brittle, they were ground in the laboratory to a fine powder which was stored in airtight containers in dark.

The determination of phytochemicals such as Tannins, Saponins, Flavonoids, Alkaloids, Anthocyanins, Phenols, were gravimetrically done using the methods already described by Harbone (1973).

Similarly, the determination of mineral elements was carried out using methods previously described by James (1995).

Preparation of Plant Extract

Ten grams (10g) of the dry grounded leaf sample were dispensed in a beaker, and 100ml of distilled water were added and the mixture was shaken well and allowed to stand for two to four hours (2-4hrs).

Ten grams (10g) of the dry grounded leaf sample were dispensed in a second beaker, and 100ml of 99.7% Ethyl alcohol Ethanol were added and the mixture was shaken and allowed to stand for 2-4hrs.

After standing, they were filtered with Whatman filter paper (No1) into another sterilized beaker, and their filtrate was placed on the steam bath and allowed to evaporate to dryness.

Determination of Antimicrobial Susceptibility

The antimicrobial susceptibility studies of the extracts (Ethanol extract) were carried out using 6mm diameter paper disc diffusion technique previously described by (Cheesbrough 2000).

The Ethanol leaf extracts of the samples were prepared as described above. Standard concentrations of the extract were introduced into different 6mm diameter paper discs with micropipette and preserved aseptically.

The densities of the microorganisms were adjusted as per McFarland 0.5 standard and microorganisms were grown in nutrient broth for 18hrs, and transferred to Mueller Agar (MHA).

The Ethanol Extract impregnated disc were introduced into the Mueller Hinton Agar already inoculated with the test microorganisms. The plates were incubated at 37°C for 24 hours and zones of inhibition were measured and evaluated.

Table 5. Microbial characteristics and identification for bacteria test isolates.

Tests	Isolates		
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Catalase	+	+	-
Oxidase	-	+	+
Gram Reaction	+	+	-
Morphology	Cocci	Rod	Rod
Methyl Red	-	+	-
Indole	-	+	-
Urease	+	+	+
Citrate	+	+	+
H ₂ S Formation	-	-	-
Voges Proskauer	+	+	-
Gelatin	+	+	+
Glucose	+	+	-
Lactose	+	+	-
Sucrose	+	+	-

Table 6. Identification for the Germ tube positive *Candida albicans* strains isolate using sugar fermentation tests

Sugar	Fermentation test
Xylose	+
Sucrose	+
Raffinose	+
Lactose	+
Trehalose	+
Maltose	+
Galactose	+
Cellobiose	-
Actidione	+
Inositol	-
Melibiose	+

The assays which were effective were repeated for those consecutive times.

Determination of Minimum Inhibitory Concentration (MIC)

These tests were performed on the microorganisms that showed inhibition zones. The Ethanol extracts were diluted double fold (92:2) with Mueller Hinton Broth (MERCK) in a series of ten test tubes. An aliquot of 0.5ml of the microbial suspension (Mc Farland 0.5) was used. All tubes were incubated at 37°C for 48hrs. The lowest concentration/highest dilution that did not permit any visible

growth was considered as MIC. MIC was evaluated using different microbial pathogens, and results were given as the result of 48hrs incubation.

RESULTS AND DISCUSSION

Quantitative analysis of the *Loranthus Micranthus* used in this study showed that the Tanin Content was at the concentration of $0.388 \pm 0.363\%$ as Saponin Content was at $1.020 \pm 0.373\%$, Alkaloid Content was at $1.313 \pm$, Flavonoids Content was $0.466\% \pm 0.246\%$, Phenol Content was placed at $2.093 \pm 0.713\%$, as Anthocyanins recorded $0.266 \pm 0.193\%$ (Table 1). Composite analysis

Table 1. Quantitative Phytochemical determination of Ethanolic Extract of *Loranthus Micranthus*

Phytochemicals	Percentage Composition (%) \pm SD
Tannins	(0.383) \pm 0.363
Saponins	(1.020) \pm 0.373
Flavonoids	(0.466) \pm 0.246
Phenols	(2.093) \pm 0.713
Alkaloids	(1.313) \pm 0.142
Anthocyanins	(0.266) \pm 0.193

Values in percentages are mean values of triplicate reading. SD; standard deviation.

Table 2. Mineral Element Composition of *Loranthus micranthus* in Abia State, Nigeria.

Mineral Element	Percentage Composition (%) \pm SD
Calcium	(14.695) \pm 121.1
Magnesium	(16.000) \pm 17.600
Phosphorus	(104.143) \pm 100.58
Sodium	(1.409) \pm 1.173
Potassium	(190.133) \pm 59.866

Values in percentages are mean values of triplicate reading. SD; standard deviation.

Table 3. The Zones of Inhibition of the different Concentrations of Ethanolic Extract of leaves of *Loranthus micranthus*.

ORGANISMS	INHIBITION ZONES (MM)			
	Leaf extracts in different concentration			
	300mg/disc	200mg/disc	100mg/disc	50 mg/disc
<i>Staphylococcus aureus</i>	17.00	12.70	11.30	9.30
<i>Escherichia coli</i>	16.30	11.60	9.70	7.70
<i>Pseudomonas aeruginosa</i>	15.70	11.60	11.00	7.70
<i>Salmonella typhi</i>	7.80	-	-	-
<i>Candida albicans</i>	22.00	15.70	14.30	10.30
<i>Aspergillus species</i>	13.30	11.60	9.70	9.30
<i>Penicillium species</i>	14.30	11.00	10.70	8.30

(-) No zone of Inhibition, (Resistant); mm – millimeter.

also documented that the percentage presence of calcium, magnesium, phosphorus, sodium and potassium were 14.695%, 16.00%, 104.143%, 1.409% and 190.133% respectively (Table 2). The zones of inhibitions recorded at a concentration of 300mg/disc remained mathematically higher than the zones of inhibitions at other concentration less than 300mg/disc (Table 3).

In the minimum inhibitions concentration tests, at the end of 72 hours incubation period, Turbidity was observed in different tubes that contained the extracts of different dilutions. The main stock concentration of *Loranthus micranthus* extracts was calculated as 200mg/ml. the Minimum Inhibitory Concentrations (MICs) of *Loranthus micranthus* using *staphylococcus aureus*, *Escherichia coli*,

Pseudomonas aeruginosa *Salmonella typhi*, *Candida albicans*, *Aspergillus species* and *Penicillium species* calculated as >12.50 , ≥ 11.30 , ≥ 11.40 , ≥ 7.80 , >24.00 , ≥ 9.30 and 11.50mg/L respectively (Table 4).

In this study, the result obtained indicated the presence of Tannins, Flavonoids, Saponins, Phenols, alkaloids and Anthocyanins in the leaves of Ethanolic extracts of *Loranthus micranthus* leaves.

Flavonoids, Alkaloids and Tannins of plants have been associated with antimicrobial effects in various studies. They have been shown to have antidiabetic, anti-inflammatory and anticancerous and antihypertensive effects (Owoyele et al., 2002). Anthocyanins and flavonoids are constituents of herbal products used in the

Table 4. Minimum Inhibitory Concentrations (MIC) of the Ethanolic Extracts of *Loranthus micranthus* on Susceptible Fungal and Bacterial Strains.

Organisms	Leaf Extract Concentration (mg/ml)	MIC (mg/ml)
<i>Staphylococcus aureus</i>	200	≥ 12.50
<i>Escherichia coli</i>	200	≥ 11.30
<i>Pseudomonas aeruginosa</i>	200	≥ 11.40
<i>Salmonella typhi</i>	200	≥ 7.80
<i>Candida albicans</i>	200	≥ 24.00
<i>Aspergillus species</i>	200	≥ 9.30
<i>Pencillium species</i>	200	≥ 11.50

mg/ml: milligram/millimeter

remedies of thyroid and hormonal disorders (Vivenathan, 1995).

The extract of the *Loranthus micranthus* at concentration 300mg/disc inhibited the growth of all the selected test organisms except the *Salmonella typhi*. This therefore shows that the phytochemicals in the extract had no inhibitory effect on *Salmonella typhi*. It might be that the *Salmonella typhi* strains used have acquired genes for resistance of L. Micranthus.

The observed antifungal and antibacterial properties corroborate its use in traditional medicine. Traditionally, the extracts of the plant are used in the treatment of boils, wounds, and sores which are caused some of the test organisms used in this study.

The leave extract inhibited the growth of *Candida albicans*, *Aspergillus species* and *Pencillium species* which are causative agents of infectious diseases as Candidiasis, Respiratory Mycosis, Vaginitis, Pelvic Inflammatory Disease etc. (Cowan, 1999).

The plant extracts (*Loranthus micranthus*) have also found use in the cure of diarrhea and dysentery (Igoli et al., 2003). The large zone of inhibition exhibited by the extract against *Staphylococcus aureus* and *Pseudomonas arniginosa* justifies their use by traditional practitioners in the treatment of sores, boils, open wounds.

The inhibition against *Escherichia coli* is the common cause of travellers diarrhea infections in man (Cowan, 1996). The low minimum inhibitory concentration exhibited by the extract against *staphylococcus aureus* and *candida albicans* is of great public health significance in Health Care delivery since the extracts can be used as an alternative to orthodox. (Singlelon, 1999a). The inability of the extract to inhibit *Salmonella typhi* below concentration of 300mg/disc may be that the pathogen posses another mechanism other than acquisition of resistance plasmid for detoxifying the active ingredients of the extract. The act that some micro organisms posses diverse mechanisms by

which they convert substances that inhibit their growth to non-toxic compounds supports this hypothesis (Singlelon, 1999b). A wide range of microorganisms were inhibited by the extract at different minimum inhibitory concentrations. These observations are indications that the plant *Loranthus micranthus* will lend itself for the eradication hospital strains of microbial pathogens which are known for multi-drug resistance.

In the midst of its good quality potentials, pharmacological studies are urgently needed for the formulation of refined therapies from this local herb which will be used in combacting different diseases the herb cures.

The minimum inhibitory concentrations documented in this study should be put into critical considerations in any pharmacological studies, as the long term interest in any Antimicrobial agent is to attack just the pathogens with little or no effect of the antimicrobial agent on the issue of the host.

The MICs recorded will attack microbial cells, leaving little or no effect on human tissues. This aspect might be subtle but it is also profound because it is in line with the general principle of selective toxicity. The phytochemicals discovery of new chemotherapeutics including antimicrobial agents.

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